Further Note on Acidity of Muscle during Maintained Contraction.

By H. E. Roaf.

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In reference to my recent paper,* in consequence of criticism received privately from Mr. Ritchie, I re-investigated the use of the manganese dioxide electrode, and discovered a fault in my technique which invalidates the conclusions drawn.

This fault was that the electrode was not shielded so as to prevent the muscle from touching a portion of the electrode with which it was not in equilibrium When the tension of the muscle increased there was a tendency for the muscle to straighten between the two points of attachment, and this lifted it, slightly bringing it into contact with a fresh surface of the electrode.

I wish, therefore, to acknowledge that the method of investigating living tissues by electrodes, described in my paper, is unsatisfactory, and the results obtained do not indicate the development of acid in the muscle.

Further Experimental Investigations on the Hypertrophy of the Sexual Glands.

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I.

In describing our experiments with partial castration of male animals we stated that very small particles of the testicle left in the body, ranging from a vestige to about half of a testicle, do not increase much (1). No hypertrophy occurred even when the operation was made at a very early age and when the experiment was continued for about eleven months, *i.e.*, till an age when the guinea-pig is already advanced about nine months in sexual maturity.

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* 'Roy. Soc. Proc.,' B, vol. 93, p. 406 (1922).

In four experiments of a first series about half, threequarters and seven-eighths of one testicle and the whole second testicle were removed in guinea pigs aged about ten to fifteen days. Two and a half months later the fragments were weighed; they represented only one-tenth to one-fifteenth of the weight of two normal testicles of control animals. But the microscopical examination showed afterwards that we had weighed also parts of the epididymis together with the testicular fragments. It is impossible to clean a small testicular fragment from the surrounding tissues without injury to it; fragments from the upper pole are embedded in fatty tissues or they are adherent to some other organ; those from the under pole are attached to the cauda epididymidis. Sometimes the presence of a testicular fragment was only revealed by microscopical examination. So we decided to calculate in the following experiments the volume of the fragments, sectioning them totally into series. The product of the number of sections and of the thickness of each section gave us the height, the greatest section the basis of a parallelopiped which the different fragments resembled naturally only more or less; the figure arrived at in such a way was reduced to three-quarters.

No doubt such a method is open to criticism; especially as it is certain that a considerable shrinkage takes place when the fragment is fixed. In one case (small ovaries and an ovarian fragment) we found that the total volume was diminished after fixation by 25 or even 35 per cent. The relation of volume to weight is about 1,000 to 1,050, according to the specific gravity of the tissues in general. The error due to not taking into consideration the exact shape of the fragment cannot be great.

The results of the calculations (made mostly by Dr. Wagner and controlled by myself) are given in the following Table:—

No.	Age or weight when operated.	Duration of the experiment.	Volume of the testicular fragment at the end of the experiment.
			cmm.
30	about 10 days	4½ months	about 20
34*	„ 2 weeks	11 „	„ 60
35*	„ 4½ months	7 „	„ 35
39	260 grm.	2 „	„ 20
90	240 „	3½ „	„ 40

* Inexact calculation as compared with the other three cases.

The figures of the fourth column are to be compared with those of the weight of two testicles of normal animals of the age at which the experimental animals were killed. The weight of the testicle in normal animals of the same age varies very much; even those of the same animal often differ considerably. The weight of two normal testicles at two months is about 1·0 to 1·5 grm., at four months about 1·8 to 2·5 grm., and in older animals about 4·0 grm. There can be no doubt, however, that in our experiments the testicular fragments represented only a small percentage of the normal total testicular volume. *That would remain true even if the error were of* 100 *or even* 200 *per cent.*

All the animals enumerated in the above Table were normally developed as concerned sexual characters.

The observations made on testicular fragments contradict other experimental facts concerning the hypertrophy of the sexual glands. First of all we know since the experiments of Ribbert (3) that after unilateral castration the retained testicle undergoes a marked hypertrophy. The results of our own experiments are given in the following Table:—

No.	Age when operated.	Duration of the experiment.	Weight of the retained testicle.	Weight of the testicles of normal animals of the same litter.
	weeks.	months.	grm.	grm.
2*	7	2½	2·65	0·86 and 1·11 (No. 3*)
94	6	5	1·8	
95	6	5	1·4	0·76 and 0·75 (No. 96)

* Weighed with the epididymis.

We see that in all the three cases observed the retained testicle attained a weight twice as great as that of a normal testicle.

Carmichael and Marshall (4) showed that the same is true for the ovary. They observed on rabbits that the ovary may hypertrophy after unilateral castration even in adult animals and without interfering with pregnancy, which was asserted by Bond to be a condition of hypertrophy. The observations of Carmichael and Marshall were confirmed lately by Arai (5) with rats operated at an age of twenty days, and by ourselves with rabbits as shown below:

No.	Age when operated.	Duration of the experiment.	Weight of the remaining ovary.	Weight of the ovaries of a normal animal of the same litter.
			mgrm.	mgrm.
117	1 month	6 months	76	37 and 47 (No. 118)
123	1 month	6 ,,	69	44 and 46 (No. 121)
128	6 weeks	6 ,,	121	73 and 79 (No. 129)

It follows from these experiments that the retained ovary had a weight of 85, 55 and 60 per cent. higher than that of a normal ovary.

Further, Carmichael and Marshall in four experiments showed that a very marked hypertrophy can take place even when only small particles of an ovary are left behind.

The differences in the degrees of hypertrophy of testicular and of ovarian fragments is so striking that we considered it necessary to repeat the ovarian experiments. Two experiments showed that the statement of Carmichael and Marshall was correct. In one of our experiments made on the rabbit one whole ovary and about threequarters of the second were removed at an age of one month. Six months later the ovarian fragment weighed 31 mgrm., whereas the ovaries of a normal animal of the same litter weighed 34 and 40 mgrm. By rough calculation from the microscopical sections we found for the fragment a volume of about 19 c.mm.; for the ovaries of about 24 and 27 c.mm. The fragment had in this way almost the weight (or the volume) of a normal ovary. In a second experiment on a rabbit which weighed about 1 kgrm., one whole ovary and half of the second were removed. Five months later the fragment was at least as big as an ovary of a normal animal of the same litter.

There can be no doubt, therefore, that testicular fragments left in the body react in a very different manner from that of a whole testicle or ovary, or of ovarian fragments.

II.

The microscopical examination of more than thirty testicular fragments showed us that the fragment undergoes very marked histological changes. There is a degeneration of the seminiferous tubules, and often an hypertrophy of the interstitial cells. The degeneration of the tubules leads after a certain time to a very marked diminution of the size of the individual tubules, and consequently to a diminution of the total volume of the fragment. Hence one might suggest that the absence of an hypertrophy in this case is to be explained by the degeneration of the seminiferous tubules; but in reality it is not so, as we can show by our own experiments.

A. On observing testicular fragments at different times after the operation, we were able to convince ourselves that the behaviour of the seminiferous tubules is very manifold. We often found all the tubules in a state of full degeneration or "backward development" (6); but in other cases we saw many tubules showing a more or less advanced spermatogenesis. One experiment on a guinea-pig is of special interest (Prot. No. 63). The animal, when operated on, weighed 140 grm. One whole testicle and the upper half of the second testicle were removed. The animal was backward in the development of the somatic sexual characters; but seven months after the operation, when the animal weighed 475 grm., such sexual characters as penis and intromittent sac with the horny styles were normal, though the latter, indeed, were not so long as in a normal animal of this age. Microscopical examination of the testicular fragment showed that only a very small number of seminiferous tubules had a much diminished diameter; most of the tubules were in full spermatogenesis or in a state of distension such as is generally seen when tubules having already produced sperms undergo desquamation and degeneration. Thus, even when most of the tubules possess a great diameter, the testicular fragment remains small. The total volume of the testicular fragment in Case 63 was about 30 c.mm. As this fragment represented originally the half of a testicle,* one would expect it to have, at an age of about seven months, a volume of about 500 c.mm. But in reality the fragment had only about 1 to 2 per cent. of the mass of two normal testicles. It is clear that the testicular fragment has not the power to hypertrophy.

B. In a previous paper (7) we mentioned experiments where the testicles were sectioned near the under pole, and only a very small part of the under pole was cut away, together with the cauda epididymidis. After this operation, almost the whole testicle is in a similar condition to that of a small fragment, or of a testicle with a ligatured vas deferans. A degeneration of the seminiferous tubules takes place. A testicle of such a kind, in which, as it was shown later by the microscopical examination, all the tubules were in the state of backward development (No. 72), weighed 160 mgrm. If we compare with this "reduced" weight or volume of the testicle of an adult guinea-pig, the volumes of our testicular fragments where the seminiferous tubules are in a state of degeneration, we find that no testicular fragment attains the reduced volume of two normal testicles, even if we take into consideration the fact that the figures in that Table always indicate a smaller volume than the actual. It is clear, therefore, that the testicular fragment does not hypertrophy.

III.

The hypertrophy of the remaining testicle after unilateral castration, as observed by different authors in man, or as studied experimentally, was declared to be a compensatory one. Stieve (8) used even this "compensatory" hypertrophy as an argument that the generative cells of the testicle are the internal secretory apparatus. According to him also, the hypertrophy of the ovary, which is likewise compensatory, supports the assumption that in this organ also the germinative part produces the hormone.

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* We are not able to indicate exactly how much of the testicle we leave in the body when cutting away a certain part of it, but this is of no importance for our argument, as in general one is apt to exaggerate the smallness of the remaining piece.

Our experiments with partial castration, as mentioned above, show that the arguments of Stieve are not justified. We have seen that a testicular fragment does not hypertrophy, that the total quantity of the generative elements in such a fragment represents only a very small part of the normal total quantity, even when the tubules of the fragment are only partly degenerated and mostly fully developed. Notwithstanding this, the sexual characters of animals with small testicular fragments can undergo development to a normal degree. From this it follows that the hypertrophy of the generative part of a sexual gland has nothing to do with its special internal secretory function for the organism as a whole.

I will not, indeed, say that it is shown by this that the generative part has no internal secretory function. Such a conclusion would not be justified. We have seen in a previous paper (7) that the hypertrophy of the interstitial cells in a testicular fragment cannot be considered as a compensatory one. If we would draw now also for the interstitial cells the conclusion that absence of compensatory hypertrophy means absence of internal secretory function, this would mean that neither the generative part nor the interstitial cells have an internal secretion. But we know that the testicle performs this function for the body as a whole. What I wish to emphasise is that the hypertrophy of the generative part of the testicle, or of the ovary, under certain conditions, as after unilateral castration, cannot be considered as proof of the internal secretion of the generative part, because this hypertrophy is not a compensatory one in the sense that diminished quantity of a tissue takes up the function of the whole and in virtue of this hypertrophies.

That increase of weight means not necessarily compensatory hypertrophy is made probable also by a series of very interesting experiments of Lauche (9) on frogs, who found that there is no hypertrophy of the remaining testicle after unilateral castration. On the contrary there was a very marked hypertrophy of a small testicular fragment after the removal of the greater part of both testicles. We thus see that in the frog the result is just the opposite to that in mammals. It seems clear enough that the behaviour of the testicle, after unilateral castration in mammals, cannot be due to any compensatory tendency.

IV.

The conclusion drawn here does not explain the difference which exists in mammals between the behaviour of a small testicular fragment and that of an ovarian fragment. But is it really certain that the increase of the testicle, or of the ovary or ovarian fragment, after diminution of the total quantity of testicular or ovarian mass in the body, is an hypertrophy? Is this assumption not an arbitrary one? I will now try to demonstrate that the observed difference in behaviour is only apparent if we assume that there is no hypertrophy, but only a quicker growth or development of the generative part of the diminished sexual gland, till the normal total quantity of one testicle, or of two normal ovaries, is attained.

The increase in weight of the normal growing testicle, or ovary, is caused, especially by the development of the generative part, till the climax is attained. This for the testicle will probably be when all the tubules are in spermatogenesis; although it seems that, in reality, there are always some tubules in the adult testicle which are not fully developed, or are in a state of backward development. Still we must assume that the testicles of an adult animal are normally never far from the theoretical climax. As concerns the ovary, it seems to be entirely different. Growth or development here means development of follicles. The quantity of ovarian mass will depend, in every case, on the relative number of developed follicles, as compared with the total number of ova which are in the ovary a source of growth, or, in other words, a source of "hypertrophy." If we now assume that increase of weight, after partial removal of one, or more than one, ovary, is caused by a quicker development of the generative part, that is, by a relatively augmented number of ova entering into follicular development up to a certain time, we can easily understand that an ovarian fragment will increase its weight in the same manner as a whole ovary after unilateral castration. In an ovarian fragment the anatomical substratum for increasing the ovarian mass beyond the normal limit till the total mass of two ovaries is attained, is always present; normally a great number of young ova and follicle wait, so to say, before entering upon active development. This source of follicular development practically will never be exhausted. It may even be that a transformation of germinal epithelium into ova is possible.

Let us compare with this the situation as shown in a testicular fragment. If increase in weight after partial castration is due to a quicker development of the generative part, all we can expect after partial castration is that the maximal development of the seminiferous tubules, as far as they are present, will be attained; no increase in mass beyond normal limits will be possible.

After fulfilment of spermatogenesis in all the tubules of the fragment there is no anatomical substratum for further increase. In other words, every cubic millimetre of testicle has its maximal potential mass, attained by means of spermatogenesis. On the contrary, a cubic millimetre of ovary has practically no limited potential mass; a given minimal volume of ovarian mass has an enormous potential volume, according to the number of ova which will develop to graafian follicles as compared with the total number of young ova.

As far as experiments have been made on adult animals, it is stated that the testicle increases its weight after unilateral castration only in a very limited degree. This is shown by the figures of Nothnagel (10). He made unilateral castration on twelve rabbits, eleven of which had a weight of more than 1·5 kgrm. The average weight of the exstirpated testicle was 0·82 grm. per kilogramme body weight; the average weight of the retained testicle was, 3 to 6 months later, 0·94 grm. per kilogramme. The absolute average figures were 1·66 grm. and 2·0 grm. We see that the increase was very small, representing only about 15 or 20 per cent., whereas in experiments on young animals a difference of about 100 per cent. between the retained testicle and that of the control animal seems to be the rule. Hence we may assume that, month by month, we approach the limit beyond which unilateral castration will cause no increase of weight in the remaining testicle.

In contrast to the observations in males, the experiments of Carmichael and Marshall on females made it probable that the ovary increases its weight enormously even in adult animals. It suffices to mention that in one of their experiments the removed ovary weighed 0·34 grm., whereas the retained ovary was found, 9 months later, to weigh as much as 0·75 grm. It is easy to explain the difference between the adult testicle and the adult ovary; in the ovary we have primordial follicles almost throughout life, *i.e.*, the anatomical substratum for hypertrophy.

Still another possibility should be discussed. It may be that by removing more than one testicle we so injure the seminiferous tubules that they lose the power of hypertrophy, although still able to fulfil spermatogenesis. I do not think that this is very probable, but the question deserves investigation. It seems very likely that in all the phenomena concerning hypertrophy of the generative part of the sexual glands some factor is involved which has not yet been taken into consideration. Arguing from experiments with simultaneous transplantation of testicle and ovary in the same animal and from experiments upon intra-testicular transplantation of the ovary, Sand (11) suggested that in every organism certain substances are to be found which are necessary for the development of the sexual glands. It is not impossible that such a factor might influence the result of partial castration.

Summary.

The fact is confirmed experimentally that after removal of one testicle or one ovary in young animals the remaining gland hypertrophies very considerably. Its weight may become twice that of a normal gland. The fact was also confirmed that small ovarian fragments remaining in the body after removal of one whole ovary and of part of the second, may hypertrophy and attain the weight of an entire ovary.

On the contrary testicular fragments remaining in the body after removal of one whole testicle and of a part of the second, do not hypertrophy. This lack of hypertrophy is not caused by the degeneration of the seminiferous tubules, as is proved by the following facts: (*a*) even when compared with a whole testicle, in which complete degeneration of the tubules was caused experimentally, the testicular fragment is still relatively very small; (*b*) even when the tubules are mostly of normal diameter the testicular fragment represents only a small percentage of the normal testicular mass.

As very small testicular fragments are sufficient for the development of those sexual characters, which depend upon the internal secretion of the testicle, it follows that the hypertrophy of the seminiferous part of the testicle after unilateral castration is not a compensatory one.

The difference between the behaviour of an ovarian and that of a testicular fragment seems to be caused by the fact that in the former the primordial follicles represent a source of hypertrophy, whereas in the second the single tubules can only attain the same climax as those in a normal testicle.

The difference between the behaviour of an entire testicle and of a testicular fragment can be explained by two different assumptions:—First, that the injury to the seminiferous tubules when a testicular fragment is produced, causes a loss of power of hypertrophy, although full spermatogenesis is still possible; secondly, that the so-called hypertrophy of the testicle is only apparent, the remaining testicle attaining the climax more rapidly. These two assumptions are still to be proved experimentally.

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The Influence of Various Conditions on the Time Relations of Tendon Reflexes in the Human Subject.

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The following observations constitute a preliminary account of an investigation into the time relations of certain reflexes in the human subject. The knee-jerk was primarily selected for study. The variability of magnitude of the knee-jerk in response to equal stimuli has been commented on by the majority of observers, and, according to Scheven,* such a variable response may occur even in the decerebrate animal. Whether variations of the irritability of the spinal reflex centre take place in the intact human subject there is no means of knowing, but study of the human responses has convinced us that by far the greatest factor in determining the variability of the knee-jerk is cortical activivity, and that, by taking suitable precautions, variability may be reduced to a negligible factor. In a

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* Scheven, 'Pflüger's Arch. f. d. ges. Physiologie,' vol. 117, p. 108 (1907).